

Fig. 4A

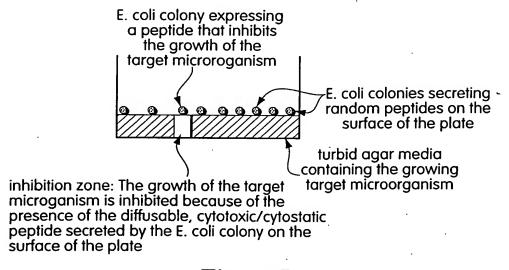


Fig. 4B



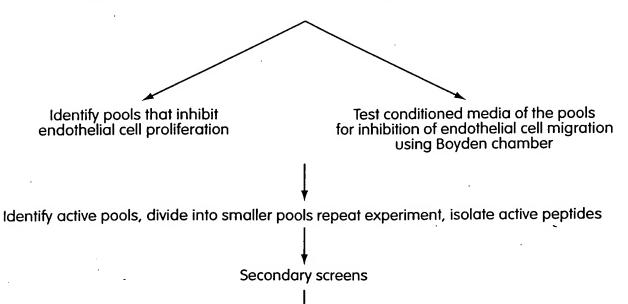
Construct a peptide library using the M13/COS phagemid vectors

Use the library in M13 peptide "display mode to isolate peptides that bind to capillary endothelial cells

Incubate the peptides that bind to endothelial cells with fibroblasts. Collect peptides that can not bind to fibroblast cells

Titrate, pool and ampilify the isolated peptides

Express the pools in COS cells ("secretion mode") using Transwells



Repeat primary and secondary screens using synthethic peptides

Test anti-angiogenic activity of the synthetic peptides in vivo

Fig. 5

JUL 2 6 2002 6/14 E. coli Stop ATTICIGAAGAGGACTIGGCACCATCACCATCACCATCIGCAGCCATIAICITGGCAGGTAAGTGCTGAGGGTGACGATCCC----TICACCTCGAAAGCAAGCTGA GAAGGAAACAGGTAAGTatgAAAAATTATTATTATTCCTTTAGTTGTTCCTTTCTATTCTCACTCCGCTGAATTACTGACATCACTTTGCCTTTCTCTCCC CAGGGGGCCACCatgaaatgcagctgggttatcttcctgatggcagtggttacaggggtcaattcagcaccaggcggatgggcggcgcgcaaaagcaaaagctc lg branch and splice acceptor Myc epitope Xbal SV40 polyA × E. coli mRNA Start lag operator ₽ Nil3pVIII ď ÷ 3 н BstXI (1) ы cleavage site (E.coli) Ö signal peptidase ы signal peptidase cleavage site ٠. ٠ S (eukaryotic) Ħ globin splice Ø z Ø lg branch and splice acceptor > pAM6 ø O 3 lac promoter plll signal peptide BstXI (2) Н IgH secretion signal (mouse) -35 box Д Σ Q ₽S Н H ď ĸ بعا 6xHis tag Ħ E. coli transciptional terminator > H H 3 × 耳 Ø E. coli Met U eukaryotic Met RBS globin splice H donor Myc epitope A Kozak 띠 lac promoter end of CMV Ø 闰

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pAM7

GGCACCCCAGGCTTTACACTTTATACTTCCGGCTCGTATATTGTGTGGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGAAAATCAAACTGGCG CGCAATTACTCTAGAGCCACCATGAAATGCAGCTGGGTTATCTTCTTCCTGATGGCAGTGGTTACAGGGGTCAATTCAGGTAAGTGAGTTAGCTCACTTA E. coli Stop SV40 polyA --TTCACCTCGAAAGCAAGCTGA Ŋ E. CO prokaryotic Starl globin splice donor signal peptidase cleavage Myc epitope site (eukaryotic) M13 pVIII z > ڻ lg branch and splice acceptor lac promoter CATCACCATCTGCAGCCATTATCTTGGCAGGTAAGTGCTGAGGGTGACGATCCC-----E. coli mRNA Start ş IgH signal peptide Α Д Ö BstXI(1) signal peptidase cleavage M S S BstXI (2) globin splice donor site (E. coli) > E. coli transciptional terminator Ig branch and splice acceptor O U eukaryotic Start × 3 "designer" secretion signal end of CMV Xbal Kozak lac operator

Fig. 7

eukaryotic Stops

X Spo

PATE	JUL 2 6 2002 &	
	rodX ↑	

pAM8

GAAGGAAAGCCACCatgTCTATCCAACACTTCCGTGTTGCATTAATCCCTTTTGCAGCGTTCTGTTTACCTGTTTTCGCAGGTCCAGGCGGATGGGCGGCGGC GAGCAAAAGCTCATTTCTGAAGAGGACTTGGCACACCATCACCATCACCATCTGCAGCCATTATCTTGGCAGGTAAGTGCTGAGGGTGACGATCCC---TTCACC BstX (1) M13 pVIII Ġ lac operator Ö E. coli/eukaryotic signal peptidase cleavage site E. coli mRNA Start globin splice > donor -10 box ы U BstXI (2) [±4 4 **B-lactamase signal sequence** Pstl lac promoter Д -35 box 6xHis tag pc; E. coli/eukaryotic Myc epitope Met Kozak end of CMV

lg branch and splice acceptor E. coli transciptional terminator 4 ĸ

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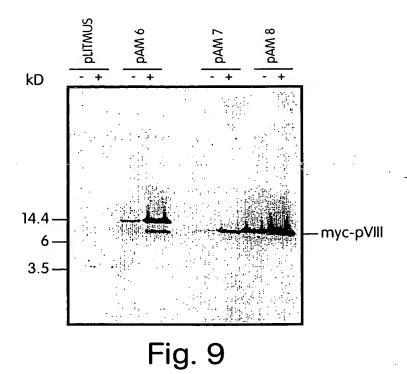
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E. coli Stop

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Characterization of the peptide display vectors

<u>Plasmids:</u>	<u>c.f.u.</u> :	<u>p.f.υ.</u> :	<u>c.f.u./p.f.u</u> .:
pAM6	2.8x10 ¹² /ml	1.9x10 ¹¹ /ml	~15
pAM7	2x10 ¹² /ml	1.5x10 ¹¹ /ml	~13
8MAq	10 ¹² /ml	2.2x10 ¹¹ /ml	~5
pLITMUS	2x10 ¹² /ml	8.4x10 ¹¹ /ml	~2

Fig.11

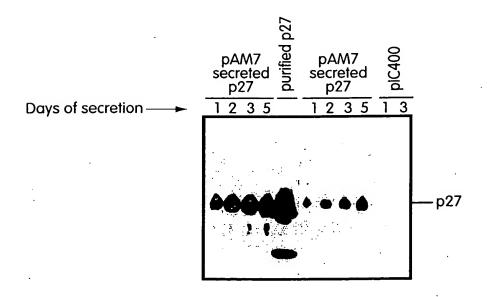


Fig. 12



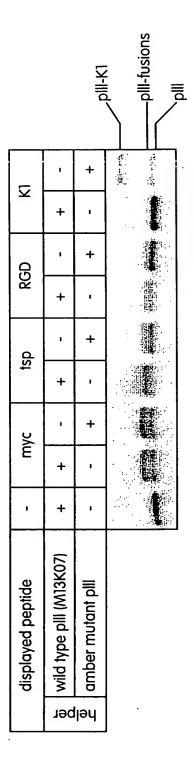


Fig. 13



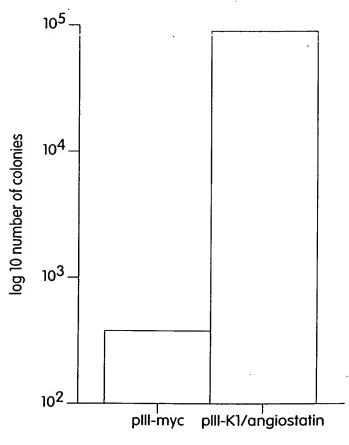


Fig. 14



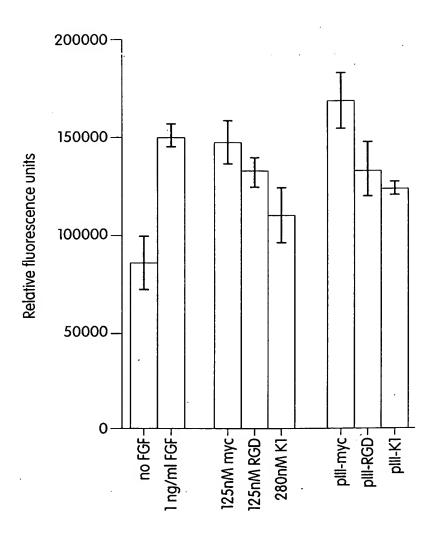


Fig. 15A



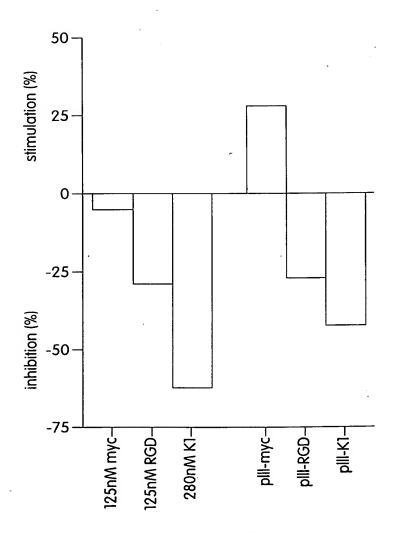


Fig. 15B